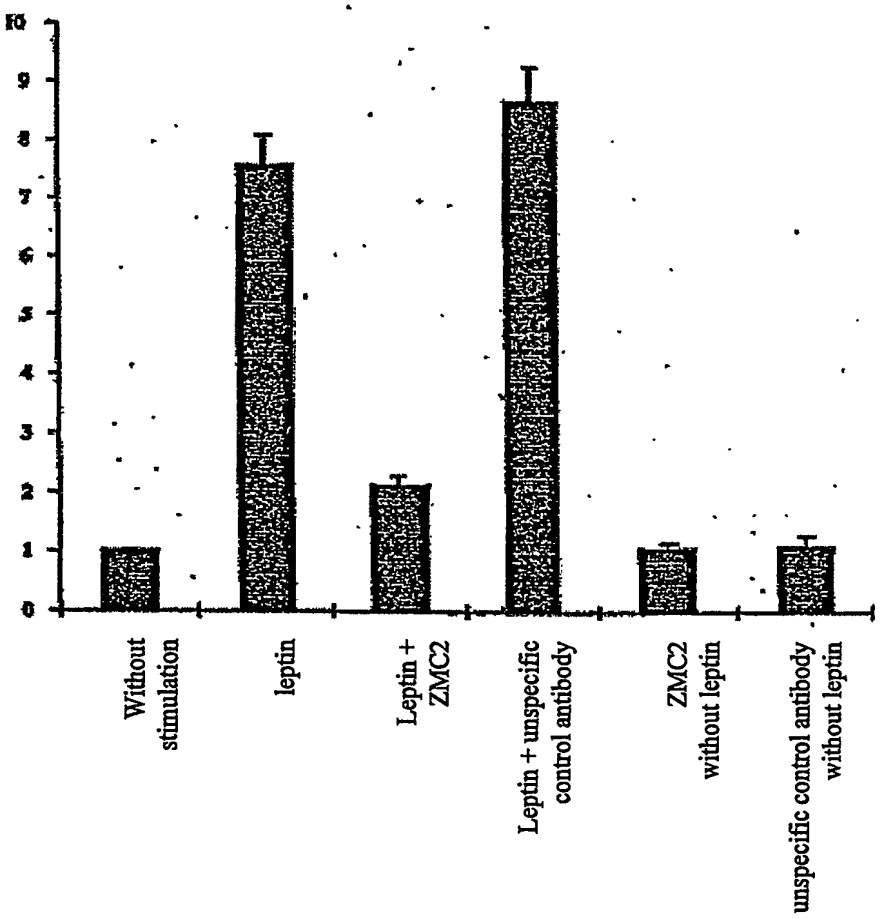

10
9
8
7
6
5
4
3
2
1
0
Without stimulation
leptin
Leptin + ZMC2
Leptin + unspecific control antibody
ZMC2 without leptin
unspecific control antibody without leptin

Figure 1

A)

```
XHNPIPMPPAAAGLLLLAAQPAMAELVMTQSPKFMSTSIGDRVNITCKAT
QNVRTAVTWYQQKPGQSPQALIFLASNRHTGVPARFTGSGSGTDFTLTIN
NVKSEDLADYFCLQHWNYPLTFGSGTKLEIKRADAAPTVSIFPPSSEQLT
SGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMS
STLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRGEC**SRVKRXQSXG
GPGTPIRPIGXPYYNSLGGGFQ
```

B)

```
DNA: NANGTCATAATCCAATACCTATGGCCTACGGCAGCCGCTGGATTGTTATTAC
 +3:   X  H  N  P  I  P  M  P  T  A  A  A  G  L  L  L  L
       pComb3 vector          SacI    VL(κ) primer
DNA: TCGCTGCCCAACCAGCCATGGCCGAGCTCGTGATGACCCAGTCTCCAAAAT
 +3:   A  A  Q  P  A  M  A  E  L  V  M  T  Q  S  P  K  F

DNA: TCATGTCCACATCAATAGGAGACAGGGTCAATATCACCTGCAAGGCCACTC
 +3:   M  S  T  S  I  G  D  R  V  N  I  T  C  K  A  T  Q

DNA: AGAATGTTCGTACTGCTGTTACCTGGTATCAACAGAAACCAGGGCAGTCTC
 +3:   N  V  R  T  A  V  T  W  Y  Q  Q  K  P  G  Q  S  P

DNA: CTCAAGCACTGATTTTCTTGGCATCCAACCGGCACACTGGTGTCCCTGCTC
 +3:   Q  A  L  I  F  L  A  S  N  R  H  T  G  V  P  A  R

DNA: GATTCACAGGCAGTGGATCTGGGACAGATTTCACTCTCACCATTAACAATG
 +3:   F  T  G  S  G  S  G  T  D  F  T  L  T  I  N  N  V

DNA: TGAAATCTGAAGACCTGGCAGATTATTTCTGTCTACAACATTGGAATTATC
 +3:   K  S  E  D  L  A  D  Y  F  C  L  Q  H  W  N  Y  P

DNA: CTCTCACGTTCGGCTCGGGGACAAAGTTGGAAATAAAACGGGCTGATGCTG
 +3:   L  T  F  G  S  G  T  K  L  E  I  K  R  A  D  A  A

DNA: CACCAACTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAG
 +3:   P  T  V  S  I  F  P  P  S  S  E  Q  L  T  S  G  G

DNA: GTGCCTCAGTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATG
 +3:   A  S  V  V  C  F  L  N  N  F  Y  P  K  D  I  N  V

DNA: TCAAGTGGAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTT
 +3:   K  W  K  I  D  G  S  E  R  Q  N  G  V  L  N  S  W
          BclI
DNA: GGACTGATCAGGACAGCAAAGACAGCACCTACAGCATGAGCAGCACCCTCA
 +3:   T  D  Q  D  S  K  D  S  T  Y  S  M  S  S  T  L  T

DNA: CGTTGACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCA
 +3:   L  T  K  D  E  Y  E  R  H  N  S  Y  T  C  E  A  T
                                                 CL(κ) primer
DNA: CTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGGGAGAGT
 +3:   H  K  T  S  T  S  P  I  V  K  S  F  N  R  G  E  C
        Stop  XbaI          NotI                  KpnI
DNA: GTTAGTAATCTAGAGTTAAGCGGCCGCAATCGAGGGGGGGCCCGGTACCCC
 +3:   *  *  S  R  V  K  R  P  Q  S  R  G  G  P  V  P  Q

DNA: AATTCGCCCTATAGGGGNGCCGTATTACAATTCACTGGGCGGCGGTTTTCA
 +3:   F  A  L  *  G  X  R  I  T  I  H  W  A  A  V  F  X

DNA: AN
 +3:
```

Figure 2

A)

LAXRGGGRKIXFXRETVIMKYLXAYGPAAGLLLLAAQPAMAQVKLLESGP
GLVAPSESLSITCTISGFSLTDDGVSWIRQPPGKGLEWLGVIWGGGSTYF
NSLFKSRLSITRDNSKSQVFLEMDSLQTDDTAMYYCAKHDGHETMDYWGQ
GTSVTVSSSKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWN
SGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTK
VDKKIVPRDCTSHHHHHH*ASLVVAVALHSFVXIKANRRPAX

B)

```
DNA: TTGGCCNCCCGCGGTGGCGGCCGCAAAATTNTATTTNCAAGGGAGACAGTC
 -1: L  A  X  R  G  G  G  R  K  I  X  F  X  R  E  T  V

DNA: ATAATGAAATACCTTTTNGCCTACGGGCCAGCCGCTGGATTGTTATTACTC
 -1: I  M  K  Y  L  X  A  Y  G  P  A  A  G  L  L  L  L
       pComb3 vector                        XhoI VHb primer
DNA: GCTGCCCAACCAGCCATGGCCCAGGTGAAACTGCTCGAGTCAGGACCTGGC
 -1: A  A  Q  P  A  M  A  Q  V  K  L  L  E  S  G  P  G

DNA: CTGGTGGCGCCCTCAGAGAGCCTGTCCATCACATGCACTATCTCAGGGTTC
 -1: L  V  A  P  S  E  S  L  S  I  T  C  T  I  S  G  F

DNA: TCATTAACCGACGATGGTGTAAGCTGGATTCGGCAGCCTCCAGGAAAGGGT
 -1: S  L  T  D  D  G  V  S  W  I  R  Q  P  P  G  K  G

DNA: CTGGAGTGGCTGGGAGTAATATGGGGTGGTGGAAGCACATACTTTAATTCA
 -1: L  E  W  L  G  V  I  W  G  G  G  S  T  Y  F  N  S

DNA: CTTTTCAAATCCAGACTGAGCATCACCAGGGACAACTCTAAGAGCCAAGTT
 -1: L  F  K  S  R  L  S  I  T  R  D  N  S  K  S  Q  V

DNA: TTCTTAGAAATGGACAGTCTACAAACTGATGACACAGCCATGTACTACTGC
 -1: F  L  E  M  D  S  L  Q  T  D  D  T  A  M  Y  Y  C

DNA: GCCAAACATGACGGACACGAGACTATGGACTATTGGGGTCAAGGAACCTCA
 -1: A  K  H  D  G  H  E  T  M  D  Y  W  G  Q  G  T  S

DNA: GTCACCGTCTCCTCATCCAAAACGACACCCCCATCTGTCTATCCACTGGCC
 -1: V  T  V  S  S  S  K  T  T  P  P  S  V  Y  P  L  A

DNA: CCTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTGGTC
 -1: P  G  S  A  A  Q  T  N  S  M  V  T  L  G  C  L  V

DNA: AAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATCCCTG
 -1: K  G  Y  F  P  E  P  V  T  V  T  W  N  S  G  S  L

DNA: TCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGACCTCTACACT
 -1: S  S  G  V  H  T  F  P  A  V  L  Q  S  D  L  Y  T

DNA: CTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTC
 -1: L  S  S  S  V  T  V  P  S  S  T  W  P  S  E  T  V

DNA: ACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATT
 -1: T  C  N  V  A  H  P  A  S  S  T  K  V  D  K  K  I
       CH1(γ1)Primer  SpeI      His tag        Stop
DNA: GTGCCCAGGGATTGTACTAGTCATCATCATCATCATCATTAAGCTAGCCTA
 -1: V  P  R  D  C  T  S  H  H  H  H  H  H  *  A  S  L

DNA: GTGGTGGCGGTGGCTCTCCATTCGTTTGTGANGATAAAGGCCAATCGNAGA
 -1: V  V  A  V  A  L  H  S  F  V  X  I  K  A  N  R  R

DNA: CCTGCNCNA
 -1: P  A  X
```

Figure 3

A)

*ATNCTTTNTTGTTCCTTTCTATGC*GGCCCAGCCGGCCATGGCCCAGGTCCAGCTG
CAGGAGTCAGGAACTGAAGTGGTAAAGCCTGGGGCTTCAGTGAAGTTGTCCT
GCAAGGCTTCTGGCTACATCTTCACAAGTTATGATATAGACTGGGTGAGGCAG
ACGCCTGAACAGGGACTTGAGTGGATTGGATGGATTTTTCCTGGAGAGGGGA
GTACTGAATACAATGAGAAGTTCAAGGGCAGGGCCACACTGAGTGTAGACAA
GTCCTCCAGCACAGCCTATATGGAGCTCACTAGGCTGACATCTGAGGACTCTG
CTGTCTATTTCTGTGCTAGAGGGGACTACTATAGGCGCTACTTTGACTTGTGGG
GCCAAGGGACCACGGTCACCGTCTCCTCATGTGGAGGCGGTTCAGGCGGAGG
TGGCTCTGGCGGTGGCGGATCTGACATTGAGCTCACCCAGTCTCCAGCAATCA
TGTCTGCATCTCCAGGGGAGAGGGTCACCATGACCTGCAGTGCCAGCTC
AAGTATACGTTACATATATTGGTACCAACAGAAGCCTGGATCCTCCCCCA
GACTCCTGATTTATGACACATCCAACGTGGCTCCTGGAGTCCCTTTTCGC
TTCAGTGGCAGTGGGTCTGGGACCTCTTATTCTCTCACAATCAACCGAAT
GGAGGCTGAGGATGCTGCCACTTATTACTGCCAGGAGTGGAGTGGTTAT
CCTCTCACGTTCGGCTCGGGCACCAAGCGGGAAATCAAACGGGCGGCCGC
AGGTGCGCCGGTGCCGTATCCGGATCCGCTGGAACCGCGT*GCCGCATAGACT-*
GTTGAA

B)

MAQVQLQESGTEVVKPGASVKLSCKASGYIFTSYDIDWVRQTPEQGLEWIG
WIFPGEGSTEYNEKFKGRATLSVDKSSSTAYMELTRLTSEDSAVYFCARG
DYYRRYFDLWGQGTTVTVSSGGGGSGGGGSGGGGSDIELTQSPAIMSASP
GERVTMTCSASSSIRYIYWYQQKPGSSPRLLIYDTSNVAPGVPFRFSGSG
SGTSYSLTINRMEAEDAATYYCQEWSGYPLTFGSGTKREIKRAAAGAPVP
YPDPLEPR

Figure 4

A)

*tcgctgcccaaccagcc***ATG**gcccaggtgaaactgctcgagtcaggacctggcctggtgg
cgccctcagagagcctgtccatcacatgcactatctcagggttctcattaaccgacgatg
gtgtaagctggattcggcagcctccaggaaagggtctggagtggctgggagtaatatggg
gtggtggaagcacatactttaattcacttttcaaatccagactgagcatcaccagggaca
actctaagagccaagttttcttagaaatggacagtctacaaactgatgacacagccatgt
actactgcgccaaacatgacggacacgagactatggactattggggtcaaggaacctcag
tcaccgtctcctcatccaaaacgacacccccatctgtctatccactggcccctggatctg
ctgcccaaactaactccatggtgaccctgggatgcctggtcaagggctatttccctgagc
cagtgacagtgacctggaactctggatccctgtccagcggtgtgcacaccttcccagctg
tcctgcagtctgacctctacactctgagcagctcagtgactgtcccctccagcacctggc
ccagcgagaccgtcacctgcaacgttgcccacccggccagcagcaccaaggtggacaaga
aaattgtgcccagggattgtactagtggtggcggaggtagtggtggcggaggtagcggtg
gcggaggttctggtggcggaggttccgaattcctcgaggtgcccatccaaaaagtccaag
atgacaccaaaaccctcatcaagacaattgtcaccaggatcaatgacatttcacacacgc
agtcagtctcctccaaacagaaagtcaccggtttggacttcattcctgggctccacccca
tcctgaccttatccaagatggaccagacactggcagtctaccaacagatcctcaccagta
tgccttccagaaacgtgatccaaatatccaacgacctggagaacctccgggatcttcttc
acgtgctggccttctctaagagctgccacttgccctgggccagtggcctggagaccttgg
acagcctgggggtgtcctggaagcttcaggctactccacagaggtggtggccctgagca
ggctgcaggggtctctgcaggacatgctgtggcagctggacctcagccctgggtgcacta
gtcatcatcatcatcatcat**TAA**gctagc*ctagtggtggcggtggctctcca*

B)

Maqvkllesgpglvapseslsitctisgfsltddgvswirqppgkglewlgviwgggsty
fnslfksrlsitrdnsksqvflemdslqtddtamyycakhdghetmdywgqgtsvtvsss
kttppsvyplapgsaaqtnsmvtlgclvkgyfpepvtvtwnsgslssgvhtfpavlqsdl
ytlsssvtvpsstwpsetvtcnvahpasstkvdkkivprdctsggggsggggsggggsgg
ggseflevpigkvqddtktliktivtrindishtqsvsskqkvtgldfipglhpiltlsk
mdqtlavyqqiltsmpsrnviqisndlenlrdllhvlafskschlpwasgletldslggv
leasgystevvalsrlqgslqdmlwqldlspgctshhhhhh

Figure 5

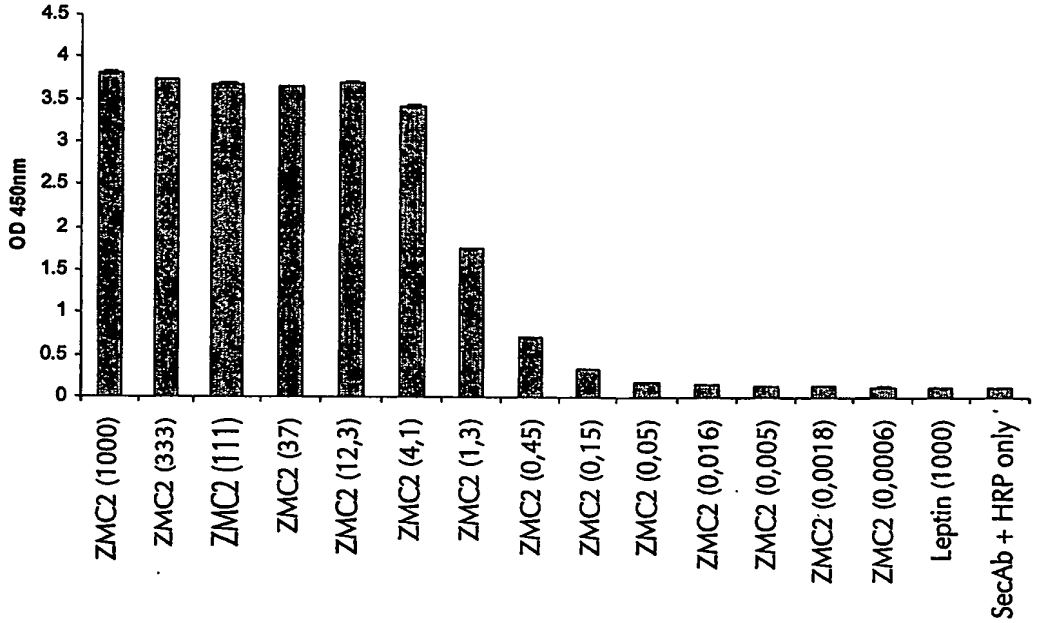

OD 450nm
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
ZMC2 (1000)
ZMC2 (333)
ZMC2 (111)
ZMC2 (37)
ZMC2 (12,3)
ZMC2 (4,1)
ZMC2 (1,3)
ZMC2 (0,45)
ZMC2 (0,15)
ZMC2 (0,05)
ZMC2 (0,016)
ZMC2 (0,005)
ZMC2 (0,0018)
ZMC2 (0,0006)
Leptin (1000)
SecAb + HRP only

Figure 6

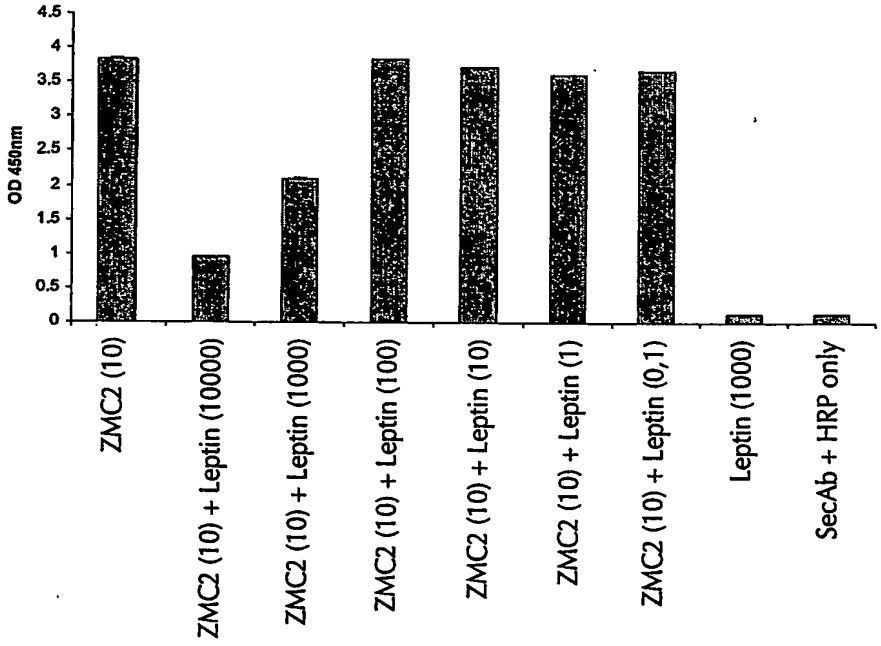

OD 450nm
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
ZMC2 (10)
ZMC2 (10) + Leptin (10000)
ZMC2 (10) + Leptin (1000)
ZMC2 (10) + Leptin (100)
ZMC2 (10) + Leptin (10)
ZMC2 (10) + Leptin (1)
ZMC2 (10) + Leptin (0,1)
Leptin (1000)
SecAb + HRP only

Figure 7

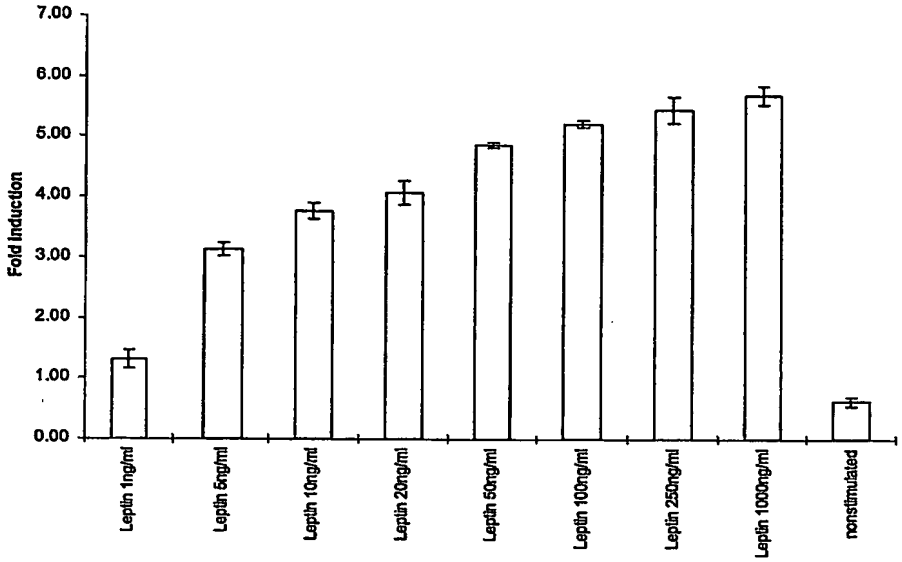

7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Fold Induction
Leptin 1ng/ml
Leptin 5ng/ml
Leptin 10ng/ml
Leptin 20ng/ml
Leptin 50ng/ml
Leptin 100ng/ml
Leptin 250ng/ml
Leptin 1000ng/ml
nonstimulated

Figure 8

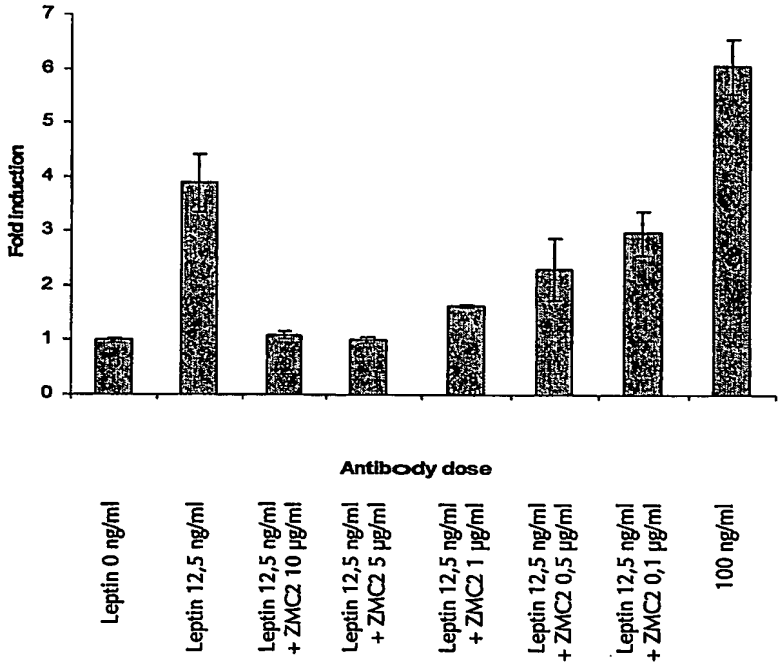

Fold induction
0
1
2
3
4
5
6
7
Antibody dose
Leptin 0 ng/ml
Leptin 12,5 ng/ml
Leptin 12,5 ng/ml + ZMC2 10 µg/ml
Leptin 12,5 ng/ml + ZMC2 5 µg/ml
Leptin 12,5 ng/ml + ZMC2 1 µg/ml
Leptin 12,5 ng/ml + ZMC2 0,5 µg/ml
Leptin 12,5 ng/ml + ZMC2 0,1 µg/ml
100 ng/ml

Figure 9

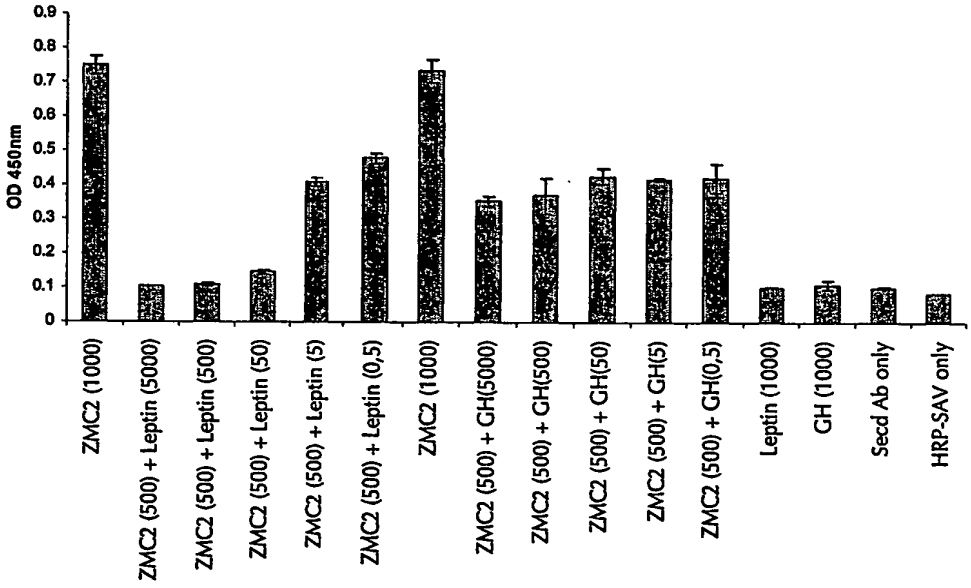

OD 450nm
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
ZMC2 (1000)
ZMC2 (500) + Leptin (5000)
ZMC2 (500) + Leptin (500)
ZMC2 (500) + Leptin (50)
ZMC2 (500) + Leptin (5)
ZMC2 (500) + Leptin (0,5)
ZMC2 (1000)
ZMC2 (500) + GH(5000)
ZMC2 (500) + GH(500)
ZMC2 (500) + GH(50)
ZMC2 (500) + GH(5)
ZMC2 (500) + GH(0,5)
Leptin (1000)
GH (1000)
Secd Ab only
HRP-SAV only

Figure 10

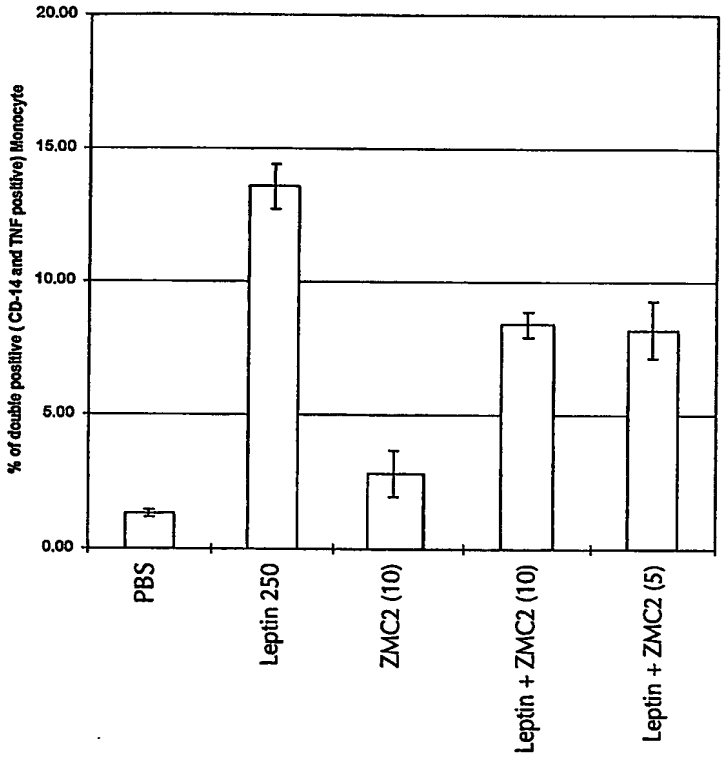

20.00
15.00
10.00
5.00
0.00
% of double positive (CD-14 and TNF positive) Monocyte
PBS
Leptin 250
ZMC2 (10)
Leptin + ZMC2 (10)
Leptin + ZMC2 (5)

Figure 11

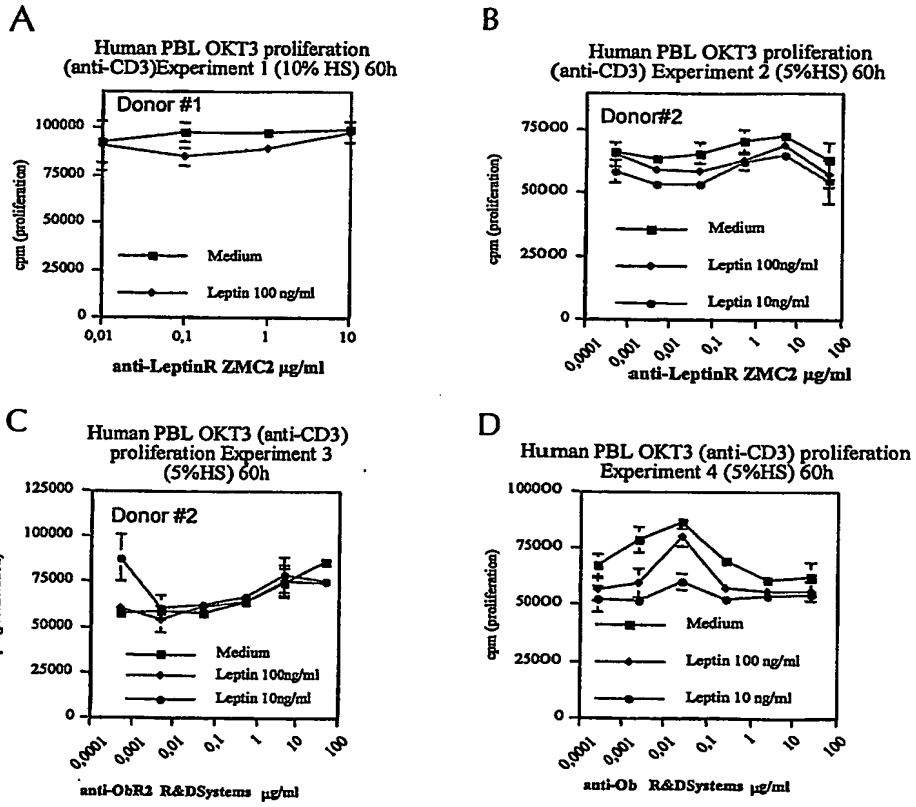

A
Human PBL OKT3 proliferation
(anti-CD3)Experiment 1 (10% HS) 60h
Donor #1
cpm (proliferation)
Medium
Leptin 100 ng/ml
anti-LeptinR ZMC2 µg/ml
B
Human PBL OKT3 proliferation
(anti-CD3) Experiment 2 (5%HS) 60h
Donor#2
cpm (proliferation)
Medium
Leptin 100ng/ml
Leptin 10ng/ml
anti-LeptinR ZMC2 µg/ml
C
Human PBL OKT3 (anti-CD3)
proliferation Experiment 3
(5%HS) 60h
Donor #2
cpm(proliferation)
Medium
Leptin 100ng/ml
Leptin 10ng/ml
anti-ObR2 R&DSystems µg/ml
D
Human PBL OKT3 (anti-CD3) proliferation
Experiment 4 (5%HS) 60h
cpm (proliferation)
Medium
Leptin 100 ng/ml
Leptin 10 ng/ml
anti-Ob R&DSystems µg/ml

Figure 12

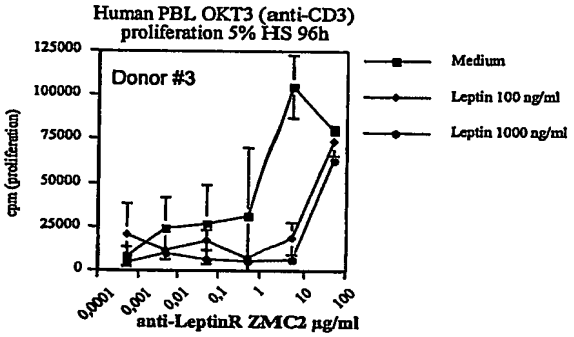

Human PBL OKT3 (anti-CD3)
proliferation 5% HS 96h
Donor #3
cpm (proliferation)
125000
100000
75000
50000
25000
0
0,0001
0,001
0,01
0,1
1
10
100
anti-LeptinR ZMC2 µg/ml
Medium
Leptin 100 ng/ml
Leptin 1000 ng/ml

Figure 13

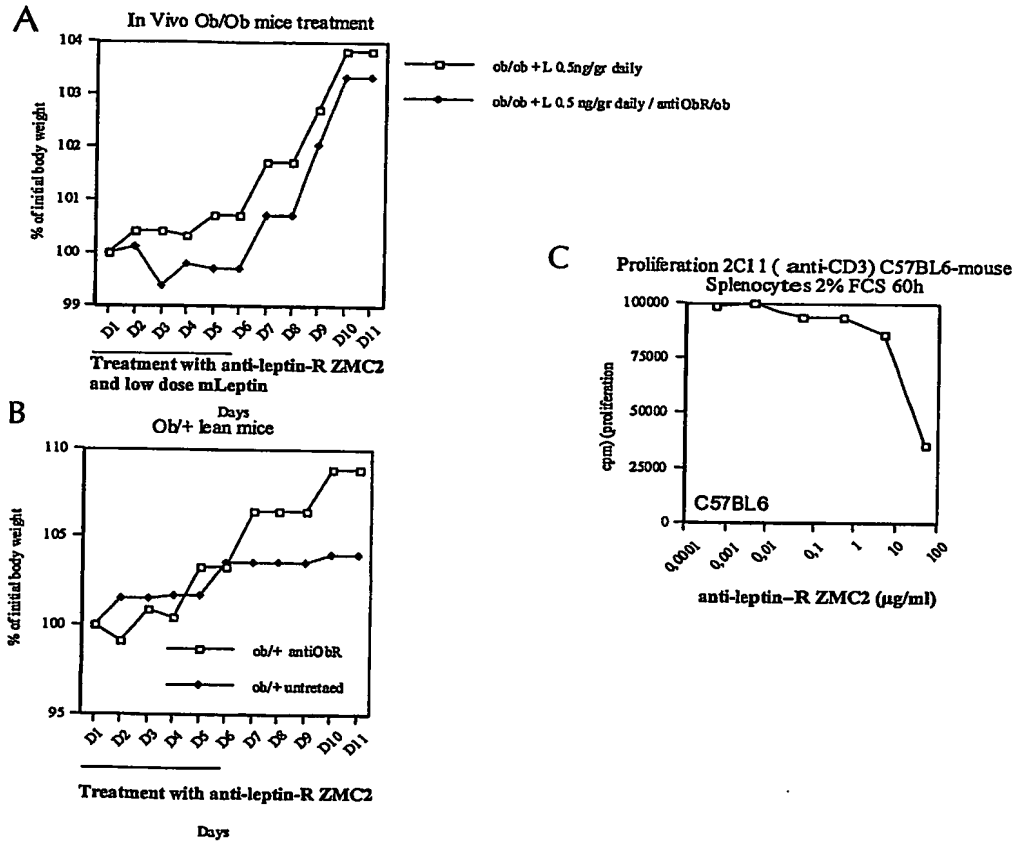

A
In Vivo Ob/Ob mice treatment
% of initial body weight
ob/ob +L 0.5ng/gr daily
ob/ob +L 0.5 ng/gr daily / antiObR/ob
Treatment with anti-leptin-R ZMC2 and low dose mLeptin
B
Ob/+ lean mice
Days
% of initial body weight
ob/+ antiObR
ob/+ untretaed
Treatment with anti-leptin-R ZMC2
Days
C
Proliferation 2C11 (anti-CD3) C57BL6-mouse Splenocytes 2% FCS 60h
cpm) (proliferation
C57BL6
anti-leptin-R ZMC2 (µg/ml)

Figure 14

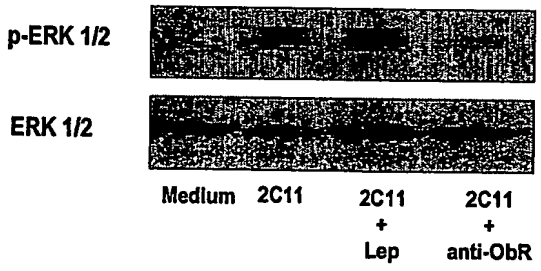


Figure 15

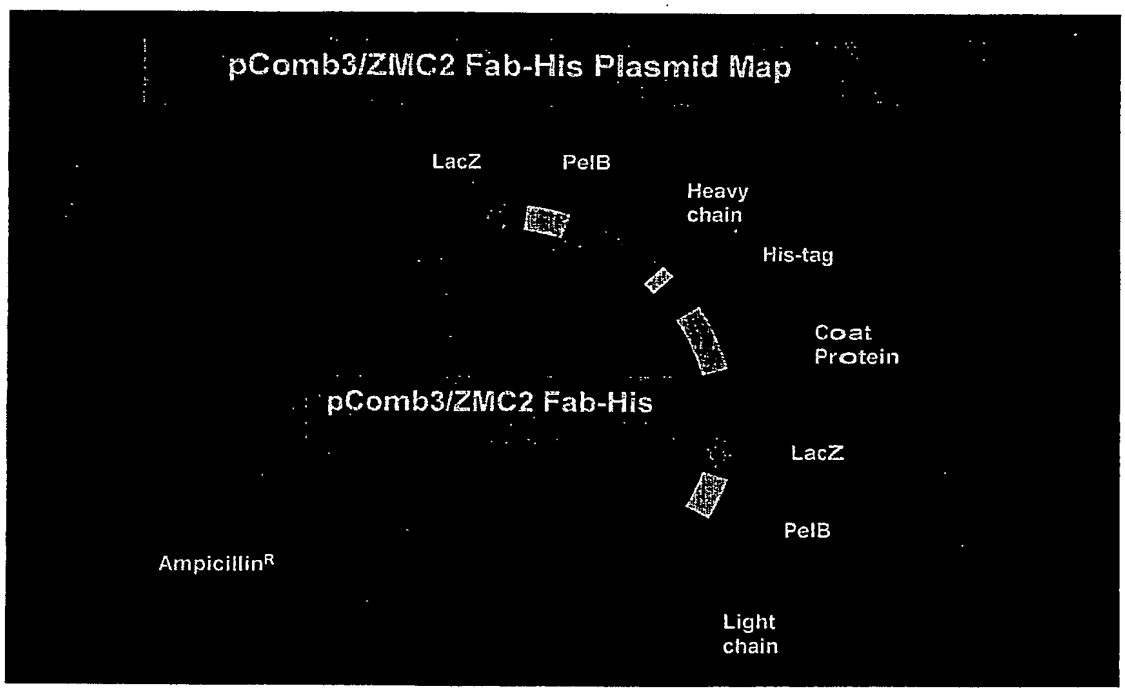

pComb3/ZMC2 Fab-His Plasmid Map
LacZ
PelB
Heavy chain
His-tag
Coat Protein
pComb3/ZMC2 Fab-His
LacZ
PelB
Ampicillin^R
Light chain

Figure 16

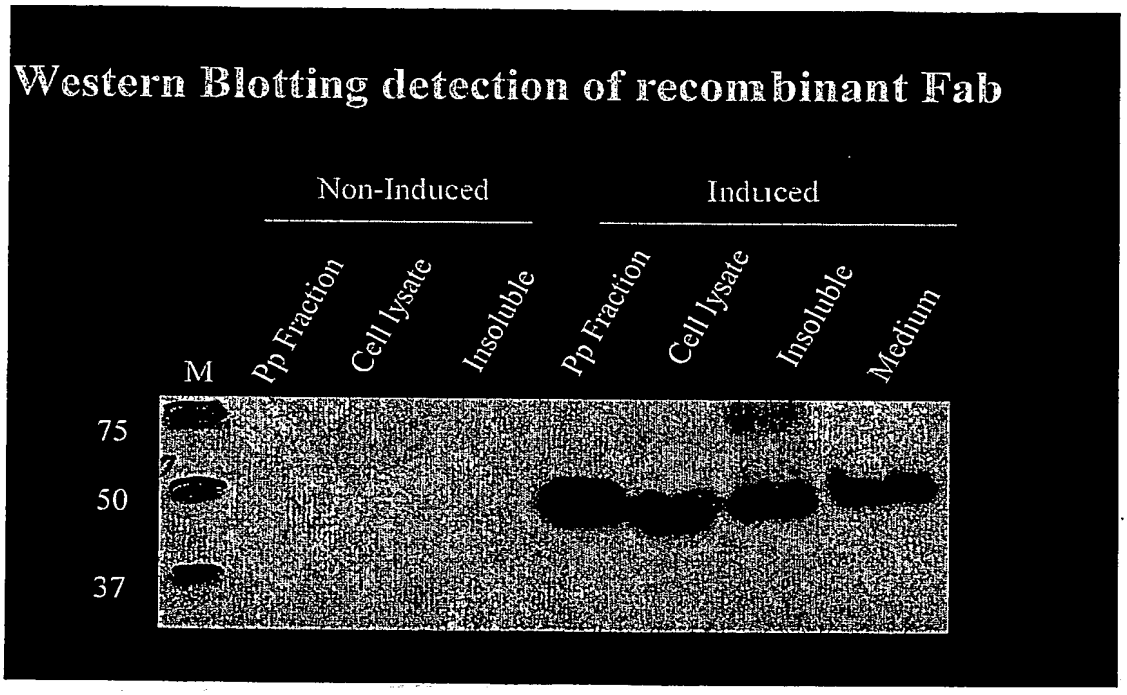

Western Blotting detection of recombinant Fab
Non-Induced
Induced
M
Pp Fraction
Cell lysate
Insoluble
Pp Fraction
Cell lysate
Insoluble
Medium
75
50
37

Figure 17

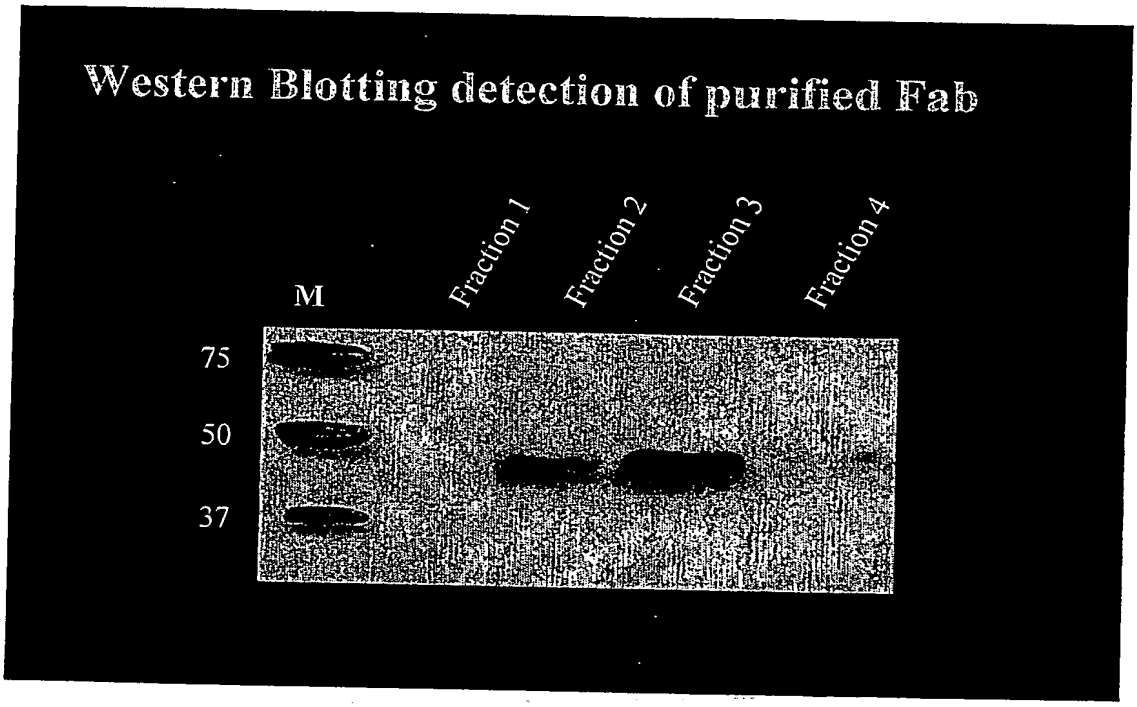

Western Blotting detection of purified Fab
M
Fraction 1
Fraction 2
Fraction 3
Fraction 4
75
50
37

Figure 18

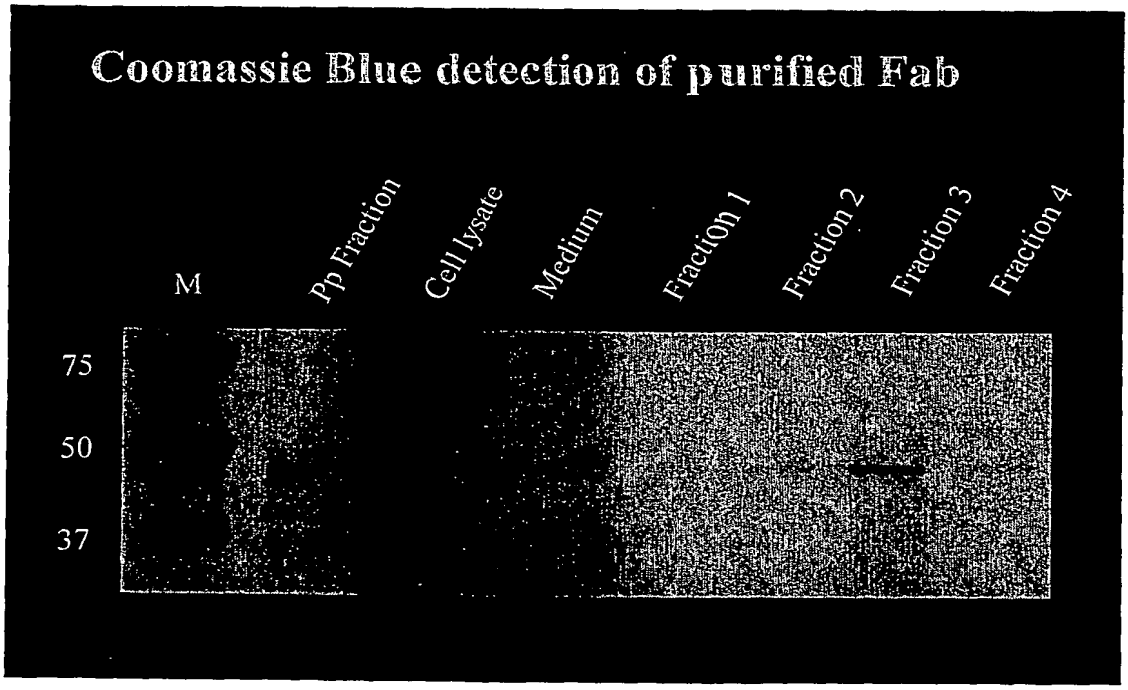
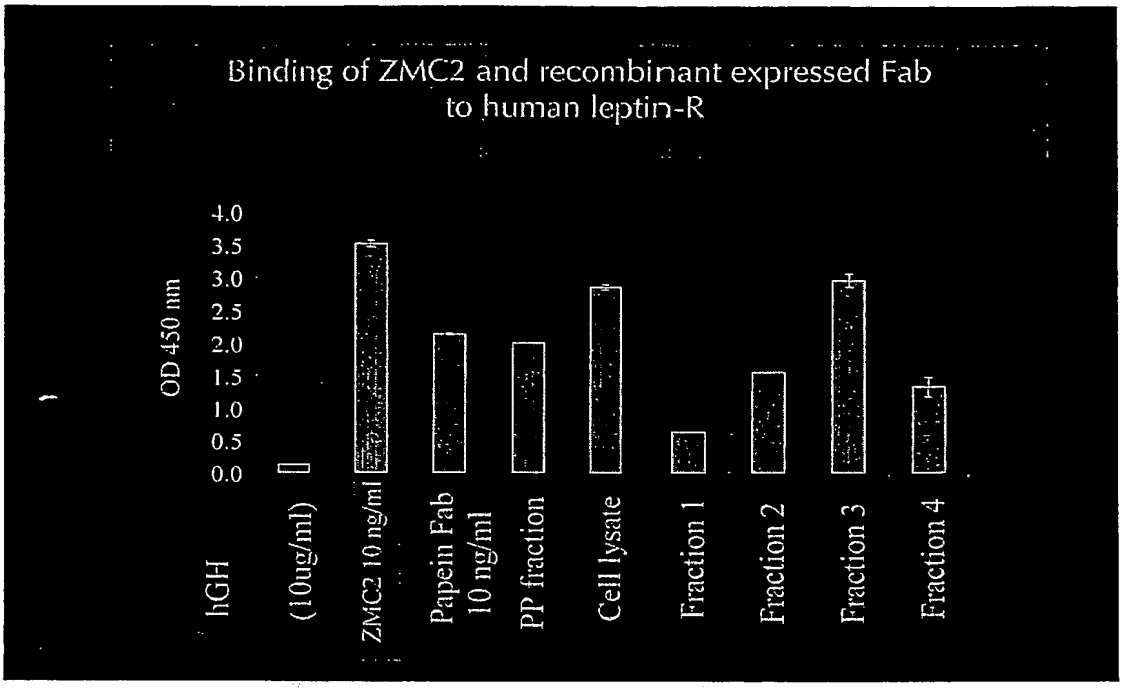

Coomassie Blue detection of purified Fab
M
Pp Fraction
Cell lysate
Medium
Fraction 1
Fraction 2
Fraction 3
Fraction 4
75
50
37

Figure 19

A)


Binding of ZMC2 and recombinant expressed Fab to human leptin-R
OD 450 nm
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
hGH (10ug/ml)
ZMC2 10 ng/ml
Papein Fab 10 ng/ml
PP fraction
Cell lysate
Fraction 1
Fraction 2
Fraction 3
Fraction 4

B)

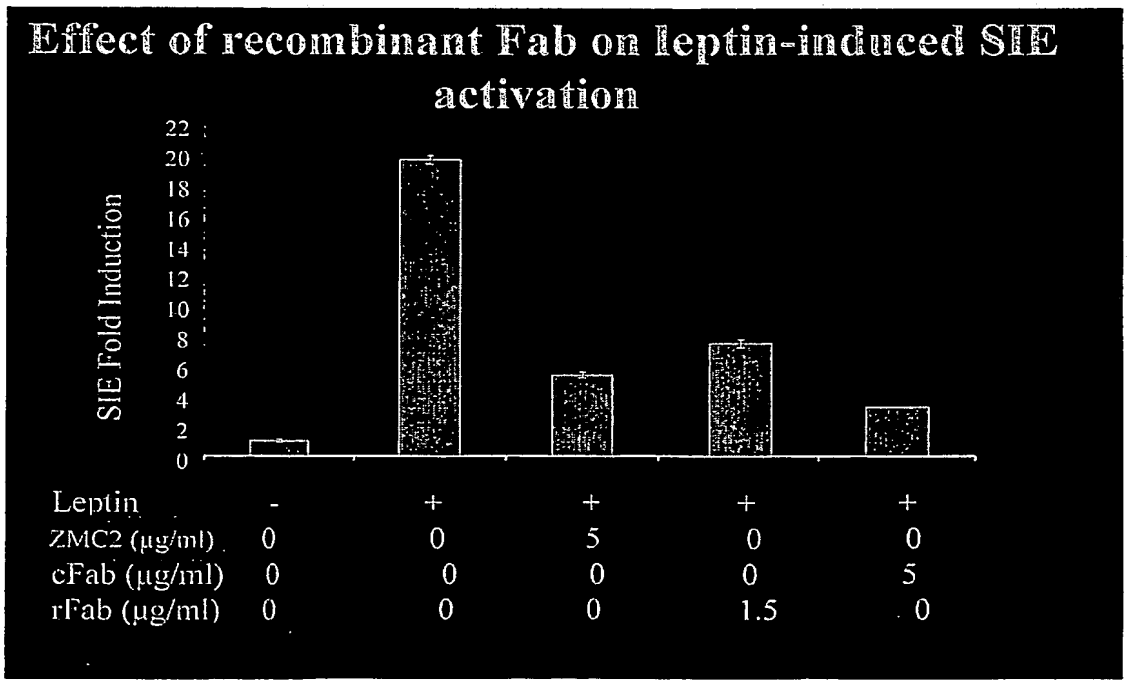

Effect of recombinant Fab on leptin-induced SIE activation
SIE Fold Induction
22
20
18
16
14
12
10
8
6
4
2
0
Leptin - + + + +
ZMC2 (µg/ml) 0 0 5 0 0
cFab (µg/ml) 0 0 0 0 5
rFab (µg/ml) 0 0 0 1.5 0

Figure 20